

Molecular basis of differential gene expression in the mouse preimplantation embryo

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Abstract

Preimplantation development of the mammalian embryo consists of stages that include formation of the zygote, blastocyst formation and implantation of the embryo into the uterus. Depending on the animal, first few cleavages of the early embryo is fully supported by translation of maternal transcripts and use of maternal proteins. After this period, the preimplantation embryo starts to transcribe from its own genome and produce products, which are necessary for further development. Eventually, differential gene expression results in production of three cell types in the preimplantation embryo; an outer transporting polarized epithelium (trophoblast) and two cell types of primitive endoderm (hypoblast), and epiblast in the inner cell mass. After implantation, the trophoblast and hypoblast give rise to extra-embryonic tissues and epiblast cells form primarily the embryo proper. Expression of maternal and embryonic transcripts and proteins, and differential expression of these products that lead to differentiation of embryonic cells are all highly coordinated events, which need to be temporally and spatially regulated during this period of development. In this review article mechanisms and paradigms that may define and regulate these cellular activities leading to the first cellular differentiation of life are presented. Considering the abundance of research data on the preimplantation development of rodents, in this review we will mainly focus on the mouse model.

Keywords: Preimplantation development; Differentiation; Signaling pathways; Chromatin remodeling.

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Overview of preimplantation development

During preimplantation development, a fertilized egg develops into a blastocyst that is able to implant into the uterine wall. Morphological changes during preimplantation development can be categorized into three main stages: increase in cell number (cleavage), cellular flattening and polarization (compaction), and production of an embryonic cavity or blastocoel (blastulation). Cleavage occurs in all preimplantation stages; however, an increase in cell number is more noticeable in the first four stages, which are the "1-cell stage" (zygote), "2-cell stage", "4-cell stage", and the "8-cell stage". During cleavage, transcription from the embry-

onic genome begins, maternal mRNA degrades to a large extent, and the control of development switches gradually from maternal to embryonic (Schultz *et al.*, 1999). During the fourth cell cycle, the cells (blastomeres) of the 8-cell stage embryo start to polarize and flatten against each other, to produce a “partially compacted 8-cell stage” embryo, which with the increase in membrane-membrane adhesion of adjacent blastomeres converts into a ball-like structure called the “fully compacted 8-cell stage” or “late 8-cell stage” (Figure 1). Since the blastomeres at the end of the fourth cell cycle undergo mitosis and cytokinesis, the 16-cell stage embryo at the beginning of fifth cell cycle appears “de-compacted”, but after cytokinesis, it converts into a fully compacted 16-cell stage embryo or “morula”. The polarized blastomeres of the 16-cell stage embryo simply divide to give rise to the two different cell types of the sixth cell cycle (Rossant and Vijn, 1980; Rossant and Tam, 2004); outer cells (future

trophoblast) and inner cell mass cells (future ICM). In the 32-cell stage morula, epithelial-type junctional complexes form between trophoblasts. When a blastocoel begins to form during the 32-cell stage, the embryo is called a blastocyst. In “early blastocysts”, the size of the blastocoel is about 1/3 of the size of the inner cell mass (ICM). The “blastocyst stage” embryo in the seventh cell cycle has approximately 64 cells and has developed a blastocoel approximately equal in size to the ICM. In addition, at this stage a differentiated layer of primitive endoderm (PE) or hypoblast has developed in the vicinity of blastocoel (Rossant and Tam, 2004). During the eighth cell cycle, in the “expanded blastocyst” the blastocoel comes to fill almost all of the internal space in the embryo. Preimplantation development concludes at this time with the release of the embryo from the zona pellucida (hatching) and its implantation into the uterine wall (Becker and Davies, 1995; Johnson, 1996).

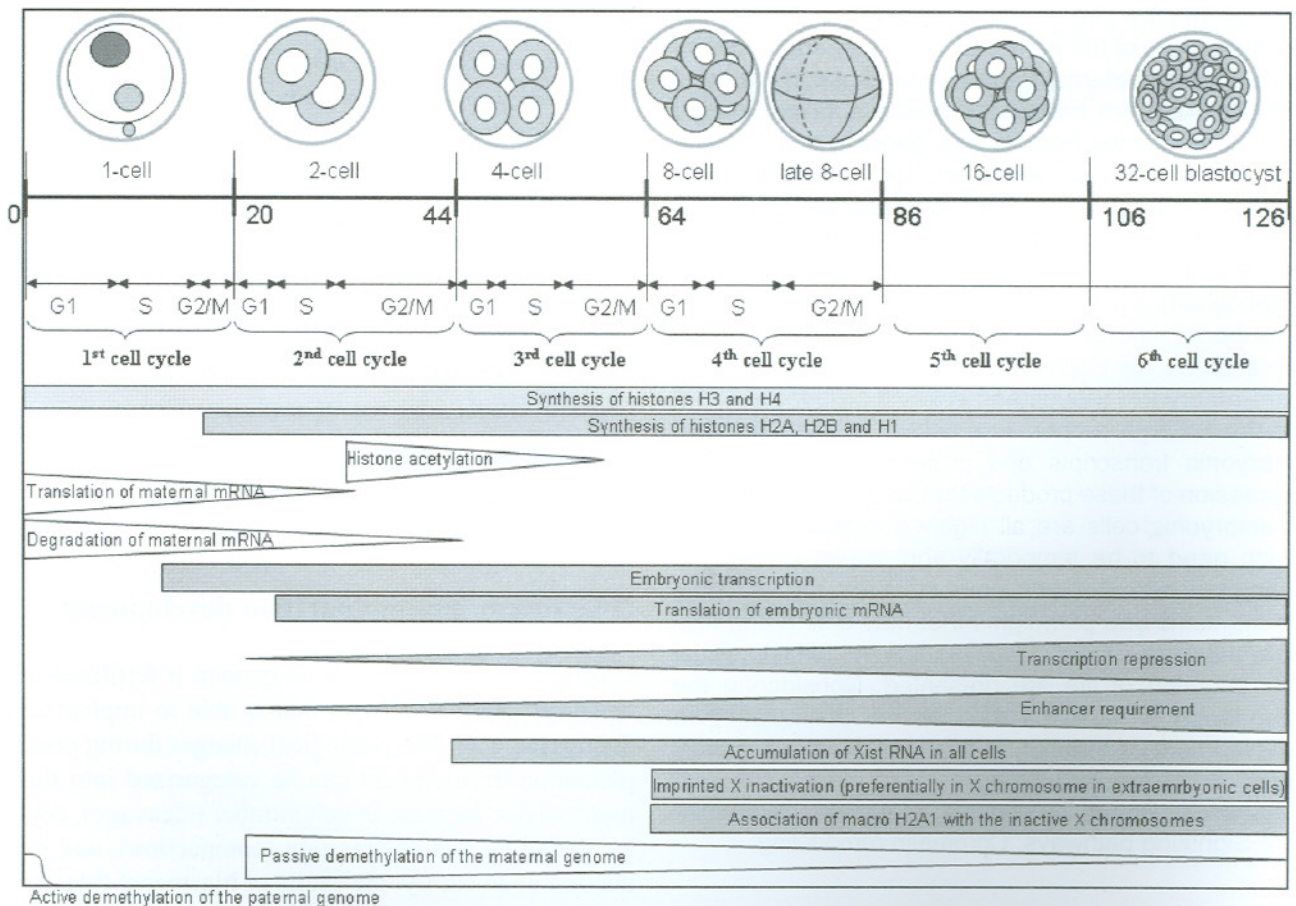


Figure 1. Major changes of transcription and chromatin biochemical structure during preimplantation mouse development. Morphologic transition of preimplantation mouse embryo has been shown at different timepoints (20, 44, 64, 86, 106, and 126 h) after fertilization. Changes in the phases of cell cycle, histone components, histone and DNA modifications, and transcription of maternal and embryonic RNA have been shown at different stages. G1, S, G2, and M denote different phases of cell cycle.

After fertilization during preimplantation development, there are three major cellular transitions. These are transition from the maternal to embryonic control of development, blastomere polarization and compaction, and blastocoel formation. In this review, the molecular basis of differential gene expression during these transitions is discussed. More specifically, the roles of chromatin remodeling and cell signaling pathways in the establishment of differential gene expression will be elaborated in detail.

Alteration of transcription and remodeling of chromatin in the early preimplantation mouse embryo

Maternal to embryonic transition of transcription:

During oocyte maturation and 12h before ovulation, the germinal vesicle breaks down, and this signals the beginning of degradation of much of the RNA that is accumulated during oocyte growth. At the same time, the rate of protein synthesis declines, due to degradation of RNA or to translational control. The time course for decay of maternal transcripts varies between genes (Gosden *et al.*, 1997). In fact, there is a complicated network of regulatory mechanisms, where stored RNAs are selectively polyadenylated for translation/degradation rather than being affected globally. Specific mRNAs are stored in the cytoplasm as mRNA-protein complexes and are isolated from the translational apparatus by masking proteins (Curtis *et al.*, 1995; Verrotti *et al.*, 1996).

The maternal to embryonic transition is the switch in control of development from products of the maternal genome to products of the embryonic genome (Telford *et al.*, 1990). While full control of development by embryonic transcripts takes at least until the blastocyst stage, the "switch" is experimentally defined as the time of the first burst of transcription from the embryonic genome. This corresponds with when development becomes sensitive to transcriptional inhibitors (Telford *et al.*, 1990). In mice the experimentally defined switch is at the early 2-cell stage. However, most proteins in the embryo will still be maternally derived at this point (Figure 1). In addition, recent works indicate that genes involved in ribosome biogenesis and assembly, protein synthesis, RNA metabolism and transcription are over-represented at the two-cell stage, suggesting that genome activation during the 2-cell stage may not be as global and promiscuous as previously proposed (Zeng and

Schultz, 2005) and not all the necessary transcripts are made by the embryonic genome.

Approximately 70-90% of the polyadenylated RNA in unfertilized eggs is lost between fertilization and the late 2-cell stage, although there is little, if any, difference in total RNA content (Piko and Clegg, 1982; Hamatani *et al.*, 2004). This indicates that much of the oocyte mRNA was for the purposes of oogenesis and the early stages of post-fertilization development. For example, it is shown that the translation of maternal RNA is required for the initiation of zygotic genome activation (Hamatani *et al.*, 2006). It also indicates possible functions for non-mRNA forms of RNA which for example may be involved in epigenetic regulations of the embryonic genome (Rassoulzadegan *et al.*, 2006). The pattern for mRNA levels of common structural and housekeeping genes is U-shaped with a nadir at the late 2-cell stage and rising concentrations after the 2-cell stage. Although, most maternal messages are gone by the end of the 2-cell stage in mice, depletion of maternal proteins occurs over the next few cleavage divisions (Kidder, 1992a; Piko *et al.*, 1984). With the expression of the embryonic genome, the maternal components that direct early development begin to be replaced (Schultz, 2002), and new products characteristic of preimplantation development appear. This has been shown by changes in the patterns of metabolically labelled proteins in high-resolution two-dimensional gel electrophoresis, during different times after fertilization. The most pronounced changes are due to the synthesis of proteins at the mid 2-cell stage, which can be inhibited by α -amanitin (Latham *et al.*, 1991). In mouse, there are other lines of evidence indicating that the one-cell embryo has potential for transcription: 1) The concentrations of the transcription factor Sp1 and the TATA box-binding protein (TBP) increase in pronuclei of one-cell embryos in a time-dependent fashion (Worrad *et al.*, 1994). 2) Functional RNA polymerase I and III are present in one-cell embryos (Nothias *et al.*, 1996). 3) 5-bromouridine 5'-triphosphate sodium (BrUTP) is incorporated into pronuclei of one-cell embryos, and incorporation is sensitive to α -amanitin and RNase treatments (Aoki *et al.*, 1997 and 2003). 4) Injection of a luciferase reporter gene under the control of the SV40 early promoter, into the male pronucleus at the early S phase results in detectable luciferase activity in G2 one-cell embryos (Ram and Schultz, 1993).

Remodeling of chromatin: Replication of DNA in the first cell cycle could facilitate the access of maternally

derived transcription factors to their cis-acting DNA-binding sequences prior to the formation of nucleosomes (Davis and Schultz, 1997). At the one-cell stage, the two haploid pronuclei enter the S phase as they migrate toward each other. Indeed, between start of DNA replication and assembly of nucleosomes, transcription factors are able to bind to DNA and start transcription (Schultz *et al.*, 1999; Schultz, 2002). Aphidicolin treatment, that inhibits entry into the S phase and DNA synthesis, decreases transcription of some transcripts e.g. eukaryotic initiation factor 1A (eIF-1A) (Davis *et al.*, 1996). This treatment also decreases BrUTP incorporation in G2 of the first cell cycle, but only by 35% (Aoki *et al.*, 1997 and 2003). This indicates that there are two classes of genes, those whose transcription is independent of DNA replication, and those whose transcription is linked to DNA replication (Schultz *et al.*, 1999; Schultz, 2002). In another words, chromatin organization is partly responsible for a transcriptionally repressive state in the 1-cell stage embryo. There also is a higher rate of BrUTP incorporation in male pronuclei, where protamines are replaced by egg histones. Similar to DNA replication, the process of histone replacement also may provide un-wrapped DNA to which transcription factors bind (McLay and Clarke, 1997). In this regard, it has been shown that a subclass of the high-mobility-group (HMG) proteins (a family of abundant low-molecular weight mammalian chromosomal proteins), HMG-I/Y, translocates into pronuclei of one-cell embryos during the first round of DNA synthesis, and that this promotes transcription (Beaujean *et al.*, 2000). HMG-I/Y may help to replace a subtype of histone H1 with histone H1^o, which accumulates in the oocyte during oogenesis (Clarke *et al.*, 1992). HMG-I/Y has high affinity for the AT-rich sequences found in scaffold or matrix-associated regions (SARs/MARs) and is able to displace histone H1 and increase chromatin accessibility (Thompson *et al.*, 1994; Thompson, 1996). The result would be an increase in the rate of transcription which by microarray data are shown to be related to 3254 genes (Hamatani *et al.*, 2004).

Other evidence for the involvement of chromatin in induction of a transcriptionally repressive state, comes from studies that have shown that the activities of promoters and replication origins from the late 1-cell stage to the 4-cell stage is repressed, and that this repression can be relieved by either sodium butyrate (inhibitor of histone deacetylase) or by enhancers (Majumder *et al.*, 1993b; Wiekowski *et al.*, 1997). Recently, it has been shown that brahma-related gene

1 (BRG1), the catalytic subunit of a chromatin remodeling complex, SWItch/Sucrose NonFermentable (SWI/SNF), is essential for maternal to embryonic transition and is derived from maternal protein stores in the oocyte (Bultman *et al.*, 2006).

The dramatic biochemical changes of chromatin have been observed during the early stages of pronuclear formation in the early preimplantation embryo and several findings point to the importance of its remodeling (Figure 1). The findings are: 1) Paternal pronuclei, in the process of replacing their sperm-specific histones with somatic histones, have higher levels of transcription than female pronuclei (Perreault, 1992; Thompson, 1996; Adenot *et al.*, 1997; Aoki *et al.*, 1997). 2) Acetylated forms of several histones (H4.Ac5, 8, 12; H3.Ac9/18 and H2A.Ac5) are transiently enriched in the nuclear periphery at the two-cell stage. This enrichment is less frequently observed in one-cell embryos and not at all at the 4-cell stage (Worrad *et al.*, 1995; Stein *et al.*, 1997; Adenot *et al.*, 1997). In contrast, H3.Ac14, H3.Ac23, H4.Ac16, and acetylated H2B are uniformly distributed throughout the nucleus (Stein *et al.*, 1997). 3) Different forms of methylated, phosphorylated, and acetylated histones H3, H4, and H2A have also been shown to stably and dynamically mark the genome of the early mouse embryo (Sarmiento *et al.*, 2004). 4) Somatic histone H1 is first detectable at the 4-cell stage (Clarke *et al.*, 1992) and its increase corresponds with a decrease in HMG-I/Y. 5) Promoters without enhancers of microinjected plasmid DNA are transcribed in 1-cell embryos, but strongly repressed in 2-cell embryos. This repression can be relieved by the inhibition of histone deacetylases using sodium butyrate (Wiekowski *et al.*, 1991 and 1997; Majumder *et al.*, 1993a; Nothias *et al.*, 1995). And 6) nuclei of early two-cell mouse embryos readily support normal embryonic development when transplanted into enucleated one-cell embryos, whereas nuclei from more advanced embryos do not (McGrath and Solter, 1984; Robl *et al.*, 1986; Howlett *et al.*, 1987). Aside from the above biochemical changes of chromatin, however, it remains to be demonstrated whether chromatin bears any structural changes (Dehghani *et al.*, 2005a) during the preimplantation period of development.

Signaling pathways in early preimplantation embryo

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Signaling pathways involved in the maternal to embryonic transition: Presence of proper extracellu-

lar signals is necessary to induce cells with appropriate developmental history to take a specific developmental route. Different signaling pathways have been found to be functional in the preimplantation mouse embryo including those that are related to protein kinase C (PKC), Wnt and its intracellular partners, bone morphogenetic protein (BMP) Notch (Wang *et al.*, 2004a), mitogen activated protein (MAP) kinase activated by Ras (Natale *et al.*, 2004; Paliga *et al.*, 2005; Maekawa *et al.*, 2005; Wang *et al.*, 2004b), protein kinase A (PKA), and receptor tyrosine kinase (Heo and Han, 2006).

- Mitosis promoting factor: During fertilization the metaphase-II related arrest of the oocyte is broken by fertilization. The hormonal signal stimulates the maturation promoting factor (MPF), now called the mitosis promoting factor activity, and the sperm-derived signal destroys CSF (cytostatic factor) activity. These activities are, in essence, kinase activities that regulate the meiotic cell cycle. MPF is a single protein kinase that induces mitosis. It has been shown that it is activated by dephosphorylation of tyrosine and threonine, and phosphorylation of threonine 161. Sustained phosphorylation of threonine 14 and dephosphorylation of tyrosines inactivates MPF (Whitaker, 1996). Experiments have identified a protein-serine/threonine kinase known as Mos as an essential component of CSF (Dekel, 1996). Mos is specifically synthesized in oocytes around the time of completion of meiosis I and is then required both for the increase in MPF activity during meiosis II and for the maintenance of MPF activity during metaphase II arrest. The downstream kinase of Mos is Rsk, which inhibits action of the anaphase-promoting complex and arrests meiosis at metaphase II. At fertilization, the increase in cytosolic Ca^{2+} signals the completion of meiosis. The anaphase-promoting complex will be activated by increase in Ca^{2+} . The resultant inactivation of MPF leads to completion of the second meiotic division, with asymmetric cytokinesis (as in meiosis I) giving rise to a second small polar body (Cooper, 2000).

- Protein kinases C and A: The role played by PKC in events associated with fertilization is controversial. A study shows that a PKC activator, 4 β -phorbol 12-myristate 13-acetate (PMA), induces Ca^{2+} oscillations in mouse oocytes (Cuthbertson and Cobbold, 1985), whereas in human oocytes, it stops the oscillations, whether added before or after sperm (Sousa *et al.*, 1996a,b; Sousa *et al.*, 1997). Calcium ion oscillations

are intracellular changes in the concentration of calcium, which is closely related to fertilization. Also, staurosporine (a PKC inhibitor) causes an increase in intracellular Ca^{2+} (Jones *et al.*, 1995; Jones, 1998). These findings indicate that PKC might be an important signaling molecule to control the levels of intracellular calcium. In the case of cortical granule (CG) release which blocks the simultaneous entry of several sperms into the oocyte (poly-spermy), it has been shown that 12-O-tetradecanoyl phorbol 13-acetate (TPA) and 1-oleyl-2-acetyl-sn-glycerol (OAG); a compound structurally similar to diacyl glycerol (DAG; one of the second messengers produced by the action of phospholipase C that leads to activation of PKC), caused CG release (Colonna and Tatone, 1993). In another study, the CG release induced by phorbol esters was blocked by PKC inhibitors, but the same inhibitors failed to have any effects on the extent of CG release caused by spermatozoa (Ducibella and LeFevre, 1997). This indicates that there is a biochemical pathway in oocytes in which PKC activation leads to CG release but that this pathway is not used by the spermatozoon at fertilization.

The role of PKC in egg activation is also disputed. One of the criteria that has been used to assess egg activation is second polar body formation. Gallicano *et al.* (1993) reported that phorbol esters can induce extrusion of a second polar body in hamster oocytes. Since up to 50% of these polar bodies resorb within 1h of addition of PMA, Moore *et al.* (1995) has argued that this may not be a bona fide polar body, because cytokinesis does not take place and its formation may be due to PKC disruption of the metaphase spindle, or to disruption of cytoskeletal structure. Gallicano *et al.* (1997) reported that PKC activation causes extrusion of the second polar body in mouse oocytes and that the polar body is resorbed after a few hours, similar to the case in hamsters. However, other studies have shown that phorbol esters do not induce second polar body formation in mouse (Cuthbertson and Cobbold, 1985; Colonna *et al.*, 1989; Moore *et al.*, 1995). In addition, Ducibella and LeFevre (1997) examined a myristoylated pseudosequence over a similar dose range to the one used by Gallicano *et al.* (1997) for inhibition of second polar body formation, and found it to be highly toxic. To date, the study of PKC at fertilization and egg activation has been limited to pharmacological manipulation that relies heavily on phorbol esters, and these agents are not specific for binding to PKC (Ahmed *et al.*, 1993; Wilkinson and Hallam, 1994; Kazanietz *et al.*, 1995). Therefore, the conclusions remain controversial.

There are several studies that suggest a role of protein phosphorylation in embryonic gene activation. An inhibitor of PKA, H8 (N-2-methylaminoethyl isoquinoline-5-sulfonamide dihydrochloride), prevents synthesis of the transcription requiring complex (TRC) (Poueymirou *et al.*, 1989). This effect of H8 on TRC synthesis is likely to be at the level of transcription, since TRC is an embryonic product of the two-cell stage embryos (Schultz, 1993). This protein is detected following *in vitro* translation of RNA obtained from 2-cell embryos, but not from one-cell or 2-cell embryos cultured in the presence of α -amanitin. Indeed, H8 and α -amanitin, have similar effects on TRC synthesis. These two compounds also inhibit the increase in heat shock protein (hsp) 70 mRNA between the one- and 2-cell stages (Manejwala *et al.*, 1991). Culture of one-cell embryos in cycloheximide under conditions that inhibit more than 95% of protein synthesis does not prevent the increase in hsp 70 mRNA, indicating that PKA affects maternally derived proteins, which are involved in transcription at the one-cell stage. Inhibitors of the calmodulin-dependent protein kinase and PKC, do not prevent embryonic gene activation (Schultz, 1993). We have shown that all of the isoforms of PKC are present between the 2-cell and blastocyst stages of mouse preimplantation development, and that each has a distinct, dynamic pattern and level of expression (Pauken and Capco, 2000; Dehghani and Hahnel, 2005). A transient increase in the nuclear concentration of PKC δ and ϵ during the early 4-cell stage has been shown to affect transcription (Dehghani *et al.*, 2005b).

Signaling pathways involved in compaction and polarization:

- E-cadherin-catenin: During the 8-cell stage, polarization is accompanied by intercellular adhesion mediated by the E-cadherin-catenin system (Larue *et al.*, 1994; Huber *et al.*, 1996). The adhesion results in compaction and formation of incomplete apicolateral junction complexes (Fleming *et al.*, 2000). Further biogenesis of the junctions, transforms the proto-epithelial phenotype of 8-cell blastomeres into the mature epithelial phenotype of trophoblast cells. Tight junctions, adherent junctions, desmosomes, and gap junctions are involved in this transformation. By approximately the 30-cell stage, functional junctional complexes have formed between apices of the outer cells of the morula. This coincides with commitment of the outer cells to become trophoblast cells as discussed above. Polarization and compaction also involve

changed distribution of cytoskeletal elements (e.g. actin filaments, microtubules), cytoplasmic organelles (e.g. endocytic vesicles), microvilli, and components of the cell cortex (e.g. actin binding proteins) (Fleming *et al.*, 1993; Fleming *et al.*, 1994). In E-cadherin null embryos, compaction occurs due to maternally inherited E-cadherin, but proper blastocysts do not form (Larue *et al.*, 1994; Kan *et al.*, 2007). Although null embryos form desmosomes and tight junctions, they cannot maintain a coherent epithelium and die at implantation. This was confirmed by treating homozygous null embryos with an antibody that blocks E-cadherin interaction and through removal of Ca^{2+} (required for E-cadherin interaction) (Riethmacher *et al.*, 1995). Together, these experiments clearly demonstrated the importance of E-cadherin in both the formation and maintenance of a polarized epithelium in the preimplantation embryo. It was hypothesized that E-cadherin induces cytocortical polarization and that this leads secondarily to polarization within the cytoplasm (Fleming *et al.*, 2001). Clayton and colleagues (1995), using inhibitors of protein and cytoskeletal assembly, showed that adhesion via E-cadherin is independent of its surface expression. This suggested that the intracellular component of E-cadherin signaling pathway was required for adhesion (Sefton *et al.*, 1996). Molecular analysis of cadherin-mediated adhesion complexes in a variety of epithelial tissues elucidated the central role of β -catenin. It not only binds to the cytoplasmic domain of E-cadherin, α -catenin and filamentous actin, but is also involved in the activation of several target genes as transcription factor (Gumbiner, 1995; Nollet *et al.*, 1999). During mouse preimplantation development, both α -catenin and β -catenin are maternally provided as proteins and mRNAs, and their transcription from the embryonic genome begins at the late 2-cell stage (Huber *et al.*, 1996). Embryos null for β -Catenin form blastocysts, implant and develop until the egg-cylinder-stage embryos (Haegel *et al.*, 1995), however, α -catenin null embryos fail to form a functional trophectoderm (Torres *et al.*, 1997).

There are several lines of evidence that emphasize the role of protein phosphorylation in post-translational modifications related to compaction and polarization. Transcription and translation of embryonic genes required for compaction take place which are then completed by the late 4-cell stage (Kidder and McLachlin, 1985; Levy *et al.*, 1986). Bloom and McConnell (1990) showed that some phosphoproteins were only found in compacted 8-cell embryos and not in other stages, suggesting a link between post-transla-

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tional mechanisms and compaction. Sefton and colleagues (1992) showed that the onset of uvomorulin phosphorylation coincides with compaction and hypothesized that this event converts uvomorulin from a non-adhesive to an adhesive form. However, cell flattening and gap junction formation take place in the absence of E-cadherin phosphorylation, and staurosporine, an inhibitor of protein kinase activity, causes premature intercellular flattening of blastomeres (O'Sullivan *et al.*, 1993). This has been further tested by using 6-dimethylaminopurine (6-DMAP), a serine-threonine kinase inhibitor that is able to induce premature cell flattening and gap junction formation at the 4-cell stage. Premature flattening was inhibited when the embryos were cultured in the presence of an anti-E-cadherin antibody or without extracellular Ca^{2+} , demonstrating that 6-DMAP-stimulated compaction requires functional E-cadherin. Although, the direct relationship of protein kinase inhibition with E-cadherin is not clear, however, it is obvious that compaction is affected by phosphorylation.

- Protein kinase C and E-cadherin: Parallel experiments with PKC, suggested that this kinase might be involved in compaction. Yamamura *et al.* (1989) found that PKC activators increased adhesion of cells in 2-, 4-, and un-compacted 8-cell embryos. Soon after, it was shown that this increased adhesion can be inhibited by a monoclonal antibody to E-cadherin. Indeed, PKC activation causes a rapid shift in the localization of E-cadherin molecules, indicating that PKC plays a role in the initiation of compaction via direct or indirect effects on E-cadherin (Winkel *et al.*, 1990). One study showed that β -catenin, a subunit of the cadherin protein complex, becomes phosphorylated during compaction, on serine/threonine residues and at the same time PKC α redistributes to contact sites as compaction initiates (Pauken and Capco, 1999), suggesting that β -catenin might be phosphorylated by this isozyme. In contrast, another study showed that β -catenin is a major tyrosine-phosphorylated protein in oocytes and early cleavage-stage embryos, and that the relative amount of phosphorylated β -catenin is greatly reduced during the morula-blastocyst transition, suggesting that tyrosine phosphorylation of β -catenin may represent a molecular mechanism to prevent E-cadherin from becoming adhesive (Ohsugi *et al.*, 1999). Additionally, a role for the myosin light-chain kinase in activation of compaction has been proposed (Kabir *et al.*, 1996). Hence, the relationship of the spatial location of a single

isozyme and a temporal event of preimplantation development can be investigated by activation/inhibition of each isozyme individually.

In conclusion, homophilic adhesion between E-cadherin molecules is a primary regulator of compaction and trophoblast differentiation. Phosphorylation /dephosphorylation reactions are important for assembly of the E-cadherin complex. Molecular partners of E-cadherin and their order of interaction during compaction remain to be identified. Since all the activators/inhibitors of PKC that have been used to date, affect the family of PKC isozymes, the role of individual isozymes in this event is obscure. Also, future experiments should determine which other cell adhesion systems are involved in compaction of preimplantation mammalian embryos.

Establishment of differential Gene expression

It is believed that the formation of different populations of cells is established during polarization and compaction of 8-cell stage embryo. The individual 1/2, 1/4, and early 1/8 blastomeres arise by approximately equal, but asynchronous cleavage divisions. They are roughly spherical, radially symmetric, have no consistent developmental fate, and are totipotent (Johnson, 1996). During the fourth cell cycle, the 1/8 blastomeres undergo a process of cellular flattening (compaction) and cellular polarization (Figure 1). These processes are the initial steps in the formation of a communicating polarized epithelium. At the end of the fourth cell cycle, the polarized blastomeres, now called polarblasts cleave to produce two-cell types in the 16-cell embryo, polarized outer cells (polarblasts), and non-polar inner cells (pluriblasts) (Johnson and McConnell, 2004; Johnson, 1996; Johnson and Selwood, 1996).

An important aspect of development is establishment of a "differential gene expression" program. While the presence of morphological differences between outer and inner cells is only noticeable for the first time at the 16-cell stage, every stage of preimplantation development displays a unique pattern of gene expression (stage-specific gene expression) (Kidder, 1992a). Transcription of several early embryonic genes has been shown to be stage-specific. For example embryonic alkaline phosphatase (EAP), histone H3, γ -actin, and connexin-43 are transcribed at the 2-cell stage. However, β -actin and transforming growth factor α (TGF- α) are not transcribed until the

4-cell stage, and glucose transporter 2 (GLUT-2) and epidermal growth factor receptor (EGF-R) until the 8-cell stage (Kidder, 1992b; Kidder, 1993). Interestingly, some genes are transiently expressed only at the 8-cell stage, U2af binding protein-related sequence (U2afbp-rs) (Latham *et al.*, 1995). U2 auxiliary factor (U2AF) is a non-snRNP (small nuclear ribonucleoprotein) protein which is required for the binding of U2 snRNP to the pre-mRNA branch site. Some genes that are transcribed during oogenesis are not transcribed during the preimplantation period, e.g. connexin-32, however, some genes are transcribed during both periods, e.g. E-cadherin. Expression of many genes that are transcribed during the cleavage stages becomes cell type-specific in the morula and blastocyst, i.e. zona occludens 1 (ZO-1; tight junction protein 1), plakoglobin (gamma-catenin, a component of desmosomes), claudin (a component of desmosomes), EAP, Na⁺-K⁺-ATPase- α , oct-4 (Octamer-4, a homeodomain transcription factor of the POU family), Mash-2 (mammalian achaete-scute homologous protein-2) (Hahnel *et al.*, 1990; Guillemot *et al.*, 1994; MacPhee *et al.*, 1994; Collins and Fleming, 1995; Yeom *et al.*, 1996; Dehghani *et al.*, 2000; Moriwaki *et al.*, 2007). Other genes are only transcribed and expressed by outer and trophoblasts later in differentiation, i.e. Desmocollin-2, and several integrins (Collins *et al.*, 1995; Sutherland and Calarco-Gillam, 1983).

Networks of epigenetic pathways directly or indirectly (through chromatin) regulate transcription. The stable determination of cell fate requires factors that initiate transcriptional patterns and mechanisms that sustain these patterns over time and through multiple cell divisions (Hagstrom and Schedl, 1997). Chromatin organization plays a role in the cellular memory that maintains stable states of transcription (Jacobs and van Lohuizen, 1999). In *Drosophila Melanogaster*, two groups of proteins, the Polycomb and trithorax groups provide transcriptional memory by "freezing" transcription states. The Polycomb group of proteins repress the expression of homeotic genes (which determine the identity of the different body segments along the anterior-posterior axis), whereas the trithorax group proteins sustain expression of these genes (Hagstrom and Schedl, 1997; Jacobs and van Lohuizen, 1999). These proteins have been shown to have the same responsibility in embryo-derived stem cells (Boyer *et al.*, 2006). In mammalian embryos, silencing and propagation of the silenced state of one of the two X chromosomes within a diploid female nucleus provides an example of tran-

scriptional cellular memory. Much attention has been focused on differential DNA methylation as a marker for imprinting and X inactivation. However, it is unclear whether DNA methylation acts as a primary determinant of differential gene activity or whether it simply reflects changes in chromatin structure that determine differential activity (Wolffe, 1996; Wolffe and Pruss, 1996). It appears that both nucleoprotein organization and acetylation patterns are important factors in the maintenance of the differential gene activity of active and inactive X chromosomes. It has been proposed that a superabundance of chromosomal proteins or transcription factors specific for large domains of DNA or individual genes, could maintain active and repressive chromatin structures during DNA replication (Wolffe, 1994).

Conclusion

Acetylation, phosphorylation, methylation and other kinds of histone modification, alteration of long-range chromatin, and stable incorporation of chromosomal proteins into the structure of DNA are the major epigenetic modifications that target DNA and program transcription. These modifications can be regulated by cell signaling pathways (Zlatanova and van Holde, 1992; Bestor *et al.*, 1994; Owen-Hughes and Workman, 1994; Edmondson and Roth, 1996; Felsenfeld *et al.*, 1996; Felsenfeld, 1996; Patterton and Wolffe, 1996; Dillon *et al.*, 1997; Elgin and Jackson, 1997; Vermaak and Wolffe, 1998; Kornberg, 1999; Schreiber and Bernstein, 2002). Presence and activity of several cell signaling components have been experimentally confirmed in the preimplantation embryo. However, it remains to be identified how different signals are orchestrated, and which signals directly create, organize, and induce the molecular program of differential gene expression.

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